

accuracy. Additionally, phenomena such as splitting events (multiple spots) within a trajectory in a single frame are naturally analyzed by our proposed method.

The model system chosen to investigate these diffusion behaviors are on glass coverslip supported phospholipid bilayers (DLPC, POPC, DMPC and DPPC). Sub-nM solutions of an amphiphilic cationic carbocyanine dye (DiI) with varying hydrophobic chain lengths are equilibrated and movies of diffusing single molecules are acquired at the lipid-water interface by TIRF-microscopy.

413-Pos

Micropatterned Model Membranes Composed of Polymerized and Fluid Lipid Bilayers

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Substrate supported planar lipid bilayers (SPBs) are versatile models of the biological membrane on solid substrates. We have developed a methodology for generating SPBs composed of polymeric and fluid phospholipid bilayers by using a photo-polymerizable diacetylene phospholipid (DynePC).⁽¹⁾ Polymeric bilayers could be generated with micro-patterns by the conventional photolithography, and the degree of polymerization could be controlled by modulating UV irradiation doses.⁽²⁾⁽³⁾ After removing non-reacted monomers, fluid lipid membranes could be integrated with polymeric bilayers. The presence of pre-formed polymeric bilayer domains enhanced the incorporation of fluid bilayer membranes into the voids between them.⁽⁴⁾ We could also immobilize biological membranes (sarcolemmal reticulum (SR) membranes from rabbit skeletal muscle) by utilizing mixtures of SR membrane with a short chain phospholipid, 1,2-hexanoyl-*sn*-glycero-3-phosphocholine (DHPC). These results clearly suggest the possibilities to reconstitute biological membranes on solid substrates for analyzing their properties in a structurally well-defined platform. In the present paper, I discuss on the unique features of the micropatterned composite model membranes and our recent approaches to construct more complex model biological membranes based on them.

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414-Pos

Self-Assembly in Phospholipid DNA - Protein Mixtures With Applications To Complex Formation in Cationic Liposome-Chromatin Systems

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We prepared complexes between cationic liposomes (CL) from a mixture of the neutral (DOPC) and the cationic (DOTAP) lipids with either nucleosome core particles (NCP) or chromatin arrays, prepared by in vitro over-expression. Microscopy showed the presence of distinct globules. Fluorescence labelling of the histone protein, DNA and lipid components showed complete co-localization under many conditions at the optical length scale, while separation of the histones from the DNA and lipids was sometimes found.

Cryo-EM confirmed array aggregates with excess of positively charged lipid forming ordered complexes of multilamellar lipids. For complexes with lower cationic charge (50% DOTAP and 50% neutral DOPC), there is an indication of less order. In most Cryo-EM samples the complexes seems to have a "subunit size" on the order of 100-200 nm, consistent with DLS data.

Synchrotron SAXS measurements were performed. The X-ray diffraction pattern demonstrates the lamellar Bragg peaks corresponding to an inter-lamellar separation of about 6-8 nm and sometimes presence of an in-plane DNA-DNA peak. This confirms a remarkably interesting phase behaviour for the system of DNA/protein (NCP or chromatin) with lipids. The SAXS and Cryo-TEM data clearly shows the formation of multilamellar aggregates with DNA sandwiched in between. This means that the DNA and the protein histone-octamer complex of the NCP and the chromatin arrays have dissociated. Hence, the question arises where the histone proteins are located, given the demonstrated co-localization at the length scale of the multilamellar complexes (a few hundred nm). One hypothesis is that the histone proteins are partially embedded in the bilayer and partially between the DNA domains. Alternatively, a few 50-100 nm size DNA-lipid complexes cluster, with histones associated in between, a type of associative phase separation.

415-Pos

Entropy Driven Structures and Interactions of Lipid Based Self Assemblies

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At present there is a surge of interest in biophysical research in elucidating collective interactions between cellular proteins, membranes and associated biomolecules leading to supramolecular structures, with the ultimate goal of relating structure to function. We present x-ray scattering data, osmotic stress experiments, cryo-electron microscopy, and optical imaging data, in self assembled systems of charged lipid bilayers and lipid-peptide complexes, which reveal unexpected structures and intermolecular forces not predicted by current electrostatic theories of charged systems. Those structures are reversible and are entropy driven due to the soft nature of the membrane interfaces. We show how membrane composition, charge density, spontaneous curvature, membrane bending rigidity and temperature control the structures and forces in those systems.

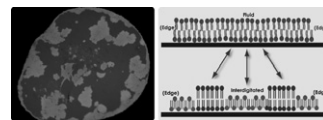
416-Pos

Frustrated Phase Transformations in Supported, Interdigitating Lipid Bilayers

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In free bilayers, the fluid to gel main phase transition of a monofluorinated phospholipid (F-DPPC) transforms a disordered fluid bilayer into a fully-interdigitated monolayer consisting of ordered acyl tails. This transformation results in an increase in molecular area and decrease in bilayer thickness. We show that when confined in patches near a solid surface, this reorganization proceeds under constraints of planar topography and total surface area. One consequence of these constraints is to limit the complete formation of the energetically-favored, interdigitated gel phase. The non-interdigitated lipids experience enhanced lateral tension, due to the expansion of the growing interdigitated phase within the constant area. The corresponding rise in equilibrium transition temperatures produces supercooled lipids that vitrify when cooled further. Ultimately, this frustrated phase change reflects a coupling between dynamics and thermodynamics, and gives rise to an unusual phase coexistence characterized by the presence of two qualitatively different gel phases.



417-Pos

Effect of Smooth Bacterial Lipopolysaccharide on the Behavior of DPPC Films

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The airspaces are lined with a DPPC-rich film called pulmonary surfactant, named for its ability to maintain normal respiratory mechanics by reducing surface tension at the air/liquid interface. Inhaled airborne particles containing smooth bacterial lipopolysaccharide (s-LPS) might incorporate into the surfactant monolayer. In this study, we evaluated the effect of s-LPS on the behavior of DPPC films by using epifluorescence microscopy combined with a surface balance. In addition, we investigated whether LPS effects could be counteracted by surfactant protein A (SP-A), which is a LPS binding protein, with the peculiarity that this protein is associated to the surfactant monolayer. Thus SP-A is in the initial defence barrier against inhaled airborne particles. Our data show that s-LPS injected in the aqueous subphase penetrated into DPPC films to form mixed DPPC/s-LPS monolayers. Low amounts of s-LPS fluidized the DPPC monolayer, as demonstrated by fluorescence microscopy and changes in the compressibility modulus. This promoted early collapse and prevented the attainment of high surface pressures. The interaction of SP-A with DPPC/s-LPS film further fluidized the monolayers and facilitated the extraction of s-LPS at surface pressures where SP-A was expelled from the mixed films, suggesting that SP-A is an LPS scavenger. A better understanding of the biophysical properties of lung surfactant monolayer and its susceptibility to LPS inhibition is important for the development of new surfactant formulations for respiratory diseases.

418-Pos

Membrane Curvature Modeling and Lipid Organization in Supported Lipid Bilayers

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